The SAUR gene family: the plant's toolbox for adaptation of growth and development

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The *SAUR* gene family: the plant's toolbox for adaptation of growth and development

Running title: *SAUR* genes ensure dynamic growth adaptation

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Highlight:

We discuss the importance of *SAUR* genes for plant growth adaptation, focussing on their molecular functions and the various mechanisms for regulation of *SAUR* activity.

Abstract

The family of Small Auxin Up-Regulated genes (SAURs) is a family of auxin-responsive genes with about 60 to 140 members in most higher plant species. Despite the early discovery of their auxin responsiveness, their function and mode of action remained unknown for a long time. In recent years, the importance of *SAUR* genes for the regulation of dynamic and adaptive growth, and the molecular mechanisms by which SAUR proteins act are increasingly understood. SAURs play a central role in auxin-induced acid growth, but can also act independently of auxin, tissue-specifically regulated by various other hormone pathways and transcription factors. In this review, we summarize the recent advances in *SAUR* gene characterization in Arabidopsis and other plant species. We particularly elaborate on their capacity to fine tune growth in response to internal and external signals, and discuss the breakthroughs in understanding the mode of action of the SAURs in relation to their complex regulation.

Introduction

The first discovery of small transcripts that rapidly responded to auxin dates back to 1987 from experiments with elongating soybean hypocotyls (McClure and Guilfoyle, 1987). In the years thereafter, these small auxin upregulated RNAs (*SAURs*) were also identified in tobacco, Arabidopsis and maize (Gil *et al.*, 1994; Knauss *et al.*, 2003; Newman *et al.*, 1993), all showing a rapid induction after auxin treatment. Both the transcript and protein half-lives were found to be very short (Knauss *et al.*, 2003; McClure and Guilfoyle, 1989; Newman *et al.*, 1993), indicating that SAUR activity can be quickly reduced after removal of the auxin stimulus, allowing very dynamic responses. Because the transcripts were identified in elongating hypocotyls and induced by the growth-hormone auxin, which had been proposed to induce cell elongation via acid growth (Rayle and Cleland, 1970; Rayle and Cleland, 1980), a link

between auxin, *SAUR* gene expression and cell elongation was apparent. However, genetic evidence demonstrating the role of SAURs in auxin-induced cell elongation remained absent for a long time.

It was the renewed interest in *SAUR* gene function in combination with a strong increase in the availability of genetic and molecular tools and resources, which recently allowed to link the SAURs to auxin-induced growth in correspondence with the acid growth theory. First, different *SAURs* were found to induce cell elongation in Arabidopsis when overexpressed (Chae *et al.*, 2012; Li *et al.*, 2015; Spartz *et al.*, 2012; Stamm and Kumar, 2013), and secondly, Spartz et al. (2014) made a major contribution to the field by showing that SAURs can interact with PP2C.D phosphatases to inhibit their activity. This inhibition prevents membrane H^+-ATPases from being dephosphorylated, which increases their activity and induces cell wall acidification. Thus, SAURs indeed induce plant growth by regulating cell wall acidification. In addition to induction by auxin, *SAURs* can be regulated by a plethora of other upstream factors, thereby regulating growth dynamically in response to internal as well as environmental cues (e.g. Favero *et al.*, 2017; Hu *et al.*, 2018; Kodaira *et al.*, 2011; Oh *et al.*, 2014; van Mourik *et al.*, 2017). Because *SAUR* overexpression is sufficient to induce growth (Fendrych *et al.*, 2016; Spartz *et al.*, 2017), other upstream factors may regulate SAUR-mediated growth independent of the auxin pathway. SAURs have thus been unveiled as growth-factors that are essential for both normal plant development as well as adaptation to environmental conditions. In the last few years, *SAUR* studies from species other than Arabidopsis have also been emerging, broadening our view on the importance of *SAURs* in the plant kingdom.

Here, we review the recent advances in *SAUR* gene characterization in Arabidopsis as well as in other plant species, and discuss their conservation and divergence in the plant kingdom. We will summarize the novel insights into the molecular function of the SAURs, and in particular elaborate on the different mechanisms of upstream and downstream regulation of *SAUR* activity, which allow the plant to fine-tune growth is a tissue-specific manner under different environmental conditions.

SAUR gene evolution in the plant kingdom

The *SAURs* form a plant-specific gene family, with the most basic members described in the moss *Physcomitrella patens*, which contains 18 *SAUR* genes (Rensing *et al.*, 2008). Notably,

the Aux/IAA-ARF-mediated auxin signalling is also present from the moss lineages to the higher plants (Lau *et al.*, 2009), suggesting that *SAUR* genes have been important for the output of the auxin response from the beginning of land plant evolution onwards. Thanks to recent advances in genome sequencing, *SAUR* families could be described in a large number of species. Besides Arabidopsis, which contains 79 SAUR genes (Ren and Gray, 2015), most higher plant species contain between 60 and 140 *SAUR* genes in their genomes, which are often arranged in clusters (Chen *et al.*, 2014; Hu *et al.*, 2018; Jain *et al.*, 2006; Li *et al.*, 2017; Wang *et al.*, 2010; Wu *et al.*, 2012). This high level of tandem and segmental duplications is remarkable, but may to some extent be explained by the small size of the SAURs, permitting duplication of the complete gene without loss of essential regions.

SAUR genes are generally intronless, with open reading frames predicted to encode proteins of a size between 7 and 20 kDa (about 60 to 180 amino acids) (Chen et al., 2014; Jain et al., 2006; Wang et al., 2010; Wu et al., 2012). These proteins have a conserved core of approximately 60 residues, whereas the homology at the N-termini and C-termini is rather low (Jain *et al.*, 2006; Park *et al.*, 2007; Ren and Gray, 2015). Within this core region, Wu et al. (2012) identified four highly conserved motifs, present in the vast majority of the SAUR proteins. The presence of these highly conserved motifs suggests that the SAUR proteins all share a conserved basic function (see below). However, their variable N- and C-termini also hint at distinct roles. For example, intracellular localization has been found to be different for the SAUR proteins (e.g. Ma *et al.*, 2017; Markakis *et al.*, 2013; Park *et al.*, 2007; Qiu *et al.*, 2013; Spartz *et al.*, 2012), and may thus be encoded by the less conserved N- or C-terminus. In addition, histidine-rich regions in the N- and C-termini of some Arabidopsis, sorghum, tomato and potato SAURs were suggested to allow metal-binding (Wu *et al.*, 2012), and some maize, Arabidopsis (SAUR70) and soybean SAURs have been shown to bind calmodulin via their N-terminus (Popescu *et al.*, 2007; Yang and Poovaiah, 2000), while many more are expected to have this capacity (Ren and Gray, 2015). The presence of the divergent N- and C-termini thus suggest functional divergence amongst the SAUR proteins.

Kodaira et al. (2011) published a phylogenetic tree of the Arabidopsis SAURs, in which three distinct SAUR clades could be recognized (indicated as clades I to III). To discuss the conservation and divergence of the SAURs in a broader perspective and evaluate the position of the Arabidopsis clades, we used the protein sequences from Arabidopsis, *Physcomitrella*, potato, tomato, rice and sorghum SAUR*s* to construct a phylogenetic tree of the SAUR family.

Based on this analysis, the plant SAUR family can be divided into three subfamilies, which all contain both monocot and eudicot sequences (see Figure 1 for an overview and Supplemental Figure S1 for the complete tree). However, all *Physcomitrella* SAURs group together in one clade of subfamily A (green in Figure 1), which consists of two *Physcomitrella* subclades that are sister to a third subclade containing SAURs from sorghum, rice, potato and Arabidopsis. These ancestral SAURs have sequences that are quite divergent from the other SAURs (see Supplementary data File 1). The other two subfamilies, B and C, have only evolved after the divergence of the mosses. These subfamilies contain clades that are lineage-specific for either higher plants, monocots, eudicots, Arabidopsis or *Solanum.* This reveals that a considerable number of recent gene duplication events have taken place throughout the evolutionary history of the *SAUR* family, and that the duplicates have often been retained. This retention may be explained by the advantage that a higher number of *SAUR* genes offers the plant. The increasing complexity of higher land plants and their capability of colonizing different habitats probably also raised a higher demand for growth adaptation in response to environmental factors such as herbivory, shade and drought. The retention of duplicated *SAUR* clusters in many different plant lineages suggests that *SAUR* copies are in general beneficial for the plant's fitness, probably enhancing the plant's options to regulate growth.

Interestingly, proteins classified into clades I and II by Kodaira et al. were recovered in two clades of subfamily C, most distantly related from the ancestral SAURs, while clade III SAURs are dispersed over many clades. The Arabidopsis SAUR63-clade, placed into clade II by Kodaira et al., can be found back as a separate clade ('clade IV') in our analysis. The SAURs from clades I and II appear to possess functions distinct from those of clade III SAURs, as many are responsive to abscisic acid (Kodaira *et al.*, 2011) and regulate cell elongation in seedlings (Sun *et al.*, 2016) (see next section). This brings forward the intriguing possibility that these functions have evolved more recently and are particularly important for the growth of higher plants.

SAUR function and mode of action

Cell elongation and growth

The long period between the discovery of auxin-upregulated RNAs and their functional characterization can be ascribed to the fact that single *SAUR* knock-outs rarely give a mutant

phenotype due to redundancy. In addition, distinct overexpression phenotypes could often only be observed after stabilization of the protein through fusion with for example GFP (Chae *et al.*, 2012; Spartz *et al.*, 2012). The first functional data therefore originated from overexpression of fusion proteins or simultaneous downregulation of a group of paralogous genes using amiRNA silencing. The majority of these studies showed that overexpression of *SAUR* genes can induce cell elongation in Arabidopsis (Bemer *et al.*, 2017b; Chae *et al.*, 2012; Franklin *et al.*, 2011; Kong *et al.*, 2013; Spartz *et al.*, 2012; Stamm and Kumar, 2013; van Mourik *et al.*, 2017). Recently, Sun et al. (2016) used a comprehensive approach to show that light-regulated seedling growth in Arabidopsis is controlled by a group of 32 redundantly acting *SAURs*. These so-called *lirSAURs* (light-induced in cotyledons and/or repressed in hypocotyls) are responsible for auxin-induced hypocotyl elongation in the dark and/or for the expansion of cotyledons upon exposure to light. Phytochrome Interacting Factors (PIFs) are important for this regulation in both tissues, but surprisingly, their breakdown upon exposure to light reduces *SAUR* expression in the hypocotyls, while inducing it in the cotyledons (Sun *et al.*, 2016). The mechanisms behind this opposite effect remain to be resolved, but different co-factors probably play a role (Sun *et al.*, 2016).

Although the function of the SAURs has thus far been mainly studied in Arabidopsis seedlings, there is increasing evidence that their cell-elongating function goes far beyond the juvenile stage, regulating growth in many different tissues. In addition to expression data, which show plant-wide *SAUR* gene activity in various species (Hu *et al.*, 2018; Jain *et al.*, 2006; van Mourik *et al.*, 2017; Wu *et al.*, 2012; Xie *et al.*, 2015), overexpression studies revealed that SAUR activity can induce growth in leaves, stems and floral organs (Chae *et al.*, 2012; Spartz *et al.*, 2012; van Mourik *et al.*, 2017). Interestingly, the specific expression of a *SAUR50*-like gene from sunflower on the east side of the stem correlates with the diurnal bending of the apex towards the sun (Atamian *et al.*, 2016), and there is evidence that the Arabidopsis *SAUR10* gene, which is upregulated in shaded conditions, affects the degree of branch bending (Bemer *et al.*, 2017b). This indicates that SAURs can also regulate light responses in the adult phase in different plant species. In conclusion, the majority of *SAUR* genes probably play a role in the induction of growth via cell elongation.

Auxin-induced cell elongation has been hypothesized to occur according to the acid growth theory, based on the observation that a low pH induces cell wall loosening (Rayle and Cleland, 1970) and that H^+ excretion takes place in response to auxin application (Rayle and

Cleland, 1980). Recently, the mechanism by which acid growth occurs via auxin and SAURs was step-by-step elucidated. First, Chen et al. (2010) showed that auxin induces phosphorylation of the plasma membrane H^+-ATPase AHA1 *in vitro*. Plasma membrane H^+-ATPases, of which *AHA1* and *AHA2* have the highest expression (Ren and Gray, 2015), require phosphorylation of the C-terminal Thr-947 residue and subsequent binding of a 14-3-3 protein for activation (Fuglsang *et al.*, 1999). Takahashi et al. (2012) then demonstrated that auxin treatment increases the phosphorylation levels and 14-3-3 binding *in planta*, without changing the amount of H^+-ATPases. The localization of SAUR19-clade proteins to the plasma membrane prompted Spartz et al. (2014) to investigate whether SAURs could regulate the H^+-ATPases, thereby discovering the link between auxin and cell membrane acidification, and achieving a major break-through in the understanding of SAUR function. In their study, Spartz et al. showed that SAUR proteins can interact with protein phosphatases of the PP2C.D family to inhibit their function. This prevents dephosphorylation of the H^+-ATPases, resulting in increased H^+-ATPase activity and induced membrane acidification (Figure 2A). Cell growth is subsequently probably achieved by activation of cell-wall expansins due to the low apoplastic pH, as well as increase of osmotic water flow due to plasma membrane hyperpolarization (Spartz *et al.*, 2017). Arabidopsis SAURs from different clades were tested for their ability to reduce PP2C.D activity *in vitro*, and they all exhibited this capacity (Spartz *et al.*, 2014; Sun *et al.*, 2016), suggesting that repression of PP2C.D activity is the general mechanism by which SAURs induce cell elongation. The Arabidopsis PP2C.D subfamily consists of nine members, of which three (D2, D5 and D6) are located to the plasma membrane. In a recent paper, Ren et al. (2018) showed that the three plasma-membrane localized PP2C.D members are the primary regulators of AHA activity *in planta*, although small contributions of the other PP2C.Ds, of which some can interact *in vitro* with SAUR19 as well, cannot be excluded. The phenotype of the *d2d5d6* triple mutant is similar to that of *SAUR* overexpression lines, with increased cell elongation in seedlings, leaves, stem and floral organs (Ren *et al.*, 2018), suggesting that the SAUR-induced cell elongation is regulated via interaction with these PP2C.Ds throughout the plant.

SAUR function in other processes

Interestingly, the overexpression lines of some *SAURs* were reported to display phenotypes other than increased cell elongation, indicating that *SAUR* family genes may perform

additional functions. Some of these functions can probably be related to their interaction with PP2C.Ds, while the mechanisms underlying other observed phenotypes may rely on different factors. In this section, we shortly discuss the involvement of SAURs in other processes based on the different phenotypes that have been reported.

An early senescence phenotype has been observed in overexpression lines of *SAUR10*, *SAUR36* and the rice gene *OsSAUR39* (Bemer *et al.*, 2017b; Hou *et al.*, 2013; Kant *et al.*, 2009), while *saur36* knock-out mutants exhibited a delayed leaf senescence phenotype (Hou *et al.*, 2013). Thus, SAURs appear to induce senescence, a function that may be regulated by interaction with a PP2C.D phosphatase, as Xiao et al. (2015) identified the PP2C.D phosphatase SENESCENCE-SUPPRESSED PROTEIN PHOSPHATASE (SSPP) as an important negative regulator of leaf senescence. SSPP (PP2C.D7 according to TAIR, but designated PP2C.D1 in Ren et al. 2018), which is mainly cytosolic localized, dephosphorylates the senescence-inducing receptor-like kinase AtSARK, localized at the plasma membrane (Figure 2B) (Xiao *et al.*, 2015). SAURs may thus interact with SSPP in the cytosol, thereby repressing its activity and activating AtSARK and leaf senescence.

Several other studies reported *SAUR* overexpression phenotypes not related to cell elongation. In particular the few studies that published about nuclear-localized SAURs report overexpression phenotypes different from cell elongation. Overexpression of *SAUR32*, the first characterized Arabidopsis *SAUR* gene, leads to reduced hypocotyl growth and abolished apical hook formation in the dark. The gene does not respond to auxin or light (Park *et al.*, 2007; Sun *et al.*, 2016) and is localized to the nucleus, suggesting that it does not interact with the plasma membrane PP2C.Ds. Overexpression of *SAUR76*, which is predominantly nuclear localized, does not promote cell elongation either, but affects the meristematic activity of the tissues, with less cells in the leaves and more cells in the roots (Markakis *et al.*, 2013). Both genes thus appear to have a function in the nucleus that may be unrelated to interaction with PP2C.Ds, or involves nuclear-localized PP2C.(D)s. Interestingly, Ma et al. (2017) reported that the cassava MeSAUR1 protein, also localized to the nucleus, can bind and regulate the promoter of the ADP glucose pyrophosphorylase subunit *MeAGPs1a*, and would thus act as a transcription factor. MeSAUR1 contains a specific N-terminus conserved in a clade of monocot and eudicot SAURs, among which the Arabidopsis SAUR10 and SAUR50 proteins (Figure 1). It is not very likely that this N-terminus provides DNA-binding activity however, as both *SAUR10* and *SAUR50* exhibit canonical cell-elongation phenotypes upon overexpression. A more

thorough *in vivo* analysis of MeSAUR1 and other SAURs in the future is required to determine whether some SAURs can act as transcription factors and to unveil the role of SAURs in the nucleus.

SAUR overexpression can also have an effect on auxin levels, polar auxin transport and/or expression of auxin pathway genes (Chae *et al.*, 2012; Kant *et al.*, 2009; Kong *et al.*, 2013; Ren and Gray, 2015; Spartz *et al.*, 2012; Xu *et al.*, 2017). Overexpression of growth-inducing *SAURs* (*SAUR19, SAUR41, SAUR63*) results in increased auxin transport, while overexpression of growth-inhibiting SAURs (*OsSAUR39, OsSAUR45*) has a repressive effect (Kant *et al.*, 2009; Xu *et al.*, 2017). These effects on the auxin pathway can be indirect, because the increase in H^+-ATPase activity probably leads to an increased plasma membrane potential, expected to induce auxin transport (Ren and Gray, 2015). However, since polar auxin transport is regulated via phosphorylation of the PIN auxin efflux carriers via PP2C.A phosphatases (Ballesteros *et al.*, 2012), one could also speculate that some SAURs might interact with other PP2C phosphatases, thereby acting directly on polar auxin transport. Another plausible explanation for the effect on polar auxin transport is the putative calmodulin-binding capacity of many SAURs, because polar auxin transport depends on calcium signalling (Vanneste and Friml, 2013; Ren and Gray, 2015).

These examples show that SAUR function is not restricted to the promotion of cell elongation. Other observed functions, such as senescence, are probably also regulated via the interaction with PP2C.Ds, while other functions may depend on other mechanisms and be more clade-specific. The presence of specific N- or C-termini could enable calmodulin-binding, metal binding (Wu *et al.*, 2012), interaction with ethylene receptors (SAUR76-78, (Li *et al.*, 2015)), or even DNA-binding capacity. The clade-specific presence of conserved N- or C-termini suggests that different sub-clades can have distinct functions. Interestingly, the Arabidopsis SAURs that can induce cell elongation and were reported by Sun et al. (2016) to be regulated during seedling morphogenesis, practically all fall into clades I and II defined by Kodaira et al. (2011), while most clade III SAURs are either not expressed in the hypocotyl/cotyledon, or do not exhibit differential expression upon transfer to the light (except for *SAUR41*, *SAUR49* and *SAUR52*) (Sun et al. 2016). This could mean that the ability to induce cell elongation, probably linked to plasma-membrane localization, is recorded in the protein sequence. Likewise, the ability to perform functions other than cell elongation may also depend on specific protein motifs. The future elucidation of protein motifs responsible

for localization and protein-protein interactions will give more insight into the possible presence of clade-specific functions.

In conclusion, the main function of SAUR proteins is the plant-wide induction of cell elongation, by repression of PP2C.D activity, in accordance with the acid growth theory. The growth-inducing function appears to be executed by plasma membrane localized SAURs interacting with PP2C.D2, D5 and D6. Furthermore, some SAURs probably perform roles in other processes than cell elongation, such as leaf senescence or cell division. In agreement with this, a number of SAURs (including MeSAUR1, OsSAUR39, OsSAUR45, SAUR32, SAUR36, SAUR40, SAUR41, SAUR55 and SAUR71) do not localize to the plasma membrane, but to the cytosol or nucleus (Kant *et al.*, 2009; Kong *et al.*, 2013; Narsai *et al.*, 2011; Park *et al.*, 2007; Qiu *et al.*, 2013; Xu *et al.*, 2017). These SAURs can possibly interact with other PP2C.Ds, which are localized to other cell compartments (Ren *et al.*, 2018) (see Figure 2), or even with PP2Cs from other classes. Interestingly, only few rice and sorghum sequences group together with the clade I and II Arabidopsis proteins, while the majority of the monocot sequences are closest to the clade III Arabidopsis proteins, of which the function appears less restricted to cell elongation. This may imply that the abundance of SAUR-proteins involved in cell elongation has evolved in the eudicots, while the majority of the monocot SAURs displays other functions.

Regulation of the different *SAURs* is highly diverse

In contrast to their general role in cell elongation, the regulation of different *SAUR* genes is highly diverse (see Figure 3 for a graphical summary). In recent years, reports from Arabidopsis as well as other species have unveiled that *SAURs* show tissue-specific expression patterns and can be regulated by a plethora of upstream factors. Although many *SAURs* can be induced by auxin (~70% in Arabidopsis (van Mourik *et al.*, 2017)), there is also a group of *SAURs*, named class II *SAURs* by Van Mourik et al. (2017), which is not responsive to auxin. At least one of these *SAURs* however (*SAUR8*), can induce cell elongation when overexpressed (van Mourik *et al.*, 2017), indicating that class II SAURs can promote growth by repressing PP2C.D activity in response to stimuli other than auxin

Factors that can up- or downregulate *SAUR* expression have been identified in different species. Characterization of the *SAUR* family in tomato (Wu *et al.*, 2012), cotton (Li

et al., 2017), poplar (Hu *et al.*, 2018), citrus (Xie *et al.*, 2015), watermelon (Zhang *et al.*, 2017), maize (Chen *et al.*, 2014) and Arabidopsis (e.g. van Mourik et al., 2017) all revealed that the different *SAUR* genes exhibit specific expression patterns throughout plant development. Moreover, the expression of different sets of *SAUR* genes can be positively or negatively regulated by many different hormones, including auxin (summarized in Ren and Gray, 2015; van Mourik et al., 2017), cytokinin (van Mourik *et al.*, 2017), gibberellic acid (GA) (Bai *et al.*, 2012; Stamm and Kumar, 2013), brassinosteroids (e.g. Oh *et al.*, 2014; van Mourik *et al.*, 2017; Wiesel *et al.*, 2015), ethylene (only *SAUR76-78* (Li *et al.*, 2015)), ABA (Kodaira *et al.*, 2011; Nemhauser *et al.*, 2006), jasmonate (JA) (Nemhauser *et al.*, 2006) as well as by different light conditions (e.g. OuYang *et al.*, 2015; Roig-Villanova *et al.*, 2007; Sun *et al.*, 2016; van Mourik *et al.*, 2017), cold (Hu *et al.*, 2018; Wu *et al.*, 2012), drought (Guo *et al.*, 2018; Wu *et al.*, 2012), high temperature (Franklin *et al.*, 2011), and high salt conditions (Guo *et al.*, 2018; Wu *et al.*, 2012) in different plant species. In general, *SAUR* genes are upregulated in response to hormones and conditions that are known to induce growth, such as auxin, brassinosteroids, gibberellin and decreased R:FR ratios, but downregulated in response to ABA, JA, and stress conditions, such as drought, cold and high salt. This stress-induced down-regulation of growth is probably compensating the plant's investment in resistance mechanisms. GUS reporter analysis revealed that the response of *SAURs* to environmental and hormonal stimuli occurs mainly in the tissue where they are already expressed (Markakis *et al.*, 2013; van Mourik *et al.*, 2017). This suggests that the tissue-specific expression of *SAUR* genes is determined by upstream transcription factors (TFs) that may be mainly developmentally regulated, while the amplitude of their expression in these tissues depends on their response to various environmental and hormonal stimuli. Plants thus contain an extensive toolbox to regulate growth dynamically in different tissues in accordance with environmental conditions.

The idea that tissue-specific *SAUR* gene expression is regulated by upstream developmentally regulated TFs is supported by large-scale ChIP-seq data, revealing frequent binding events of key developmental regulators such as LEAFY (LFY), APETALA 1 (AP1), APETALA 2 (AP2) SEPALLATA 3 (SEP3) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (van Mourik *et al.*, 2017). Induced activity of the TCP (TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL FACTOR 1) TFs TCP4 and TCP20 can also rapidly upregulate a set of *SAUR* genes (Challa *et al.*, 2016; Danisman *et al.*, 2012), but in the case of TCP4, this occurs probably indirectly via the ARF-BZR pathway (discussed below) by direct

induction of the auxin biosynthesis gene *YUCCA5* (Challa *et al.*, 2016). Only the binding and regulation of the MADS-domain TF FRUITFULL (FUL) to the *SAUR10* locus, involved in the repression of its stem-specific expression (Bemer *et al.*, 2017b), and the direct repression of *SAUR19*-clade genes by the AHL transcription factor SUPPRESSOR OF PHYTOCHROME B4-#3 (SOB3) in hypocotyls (Favero *et al.*, 2016) has been characterized in more detail so far. The factors involved in the regulation of tissue-specific *SAUR* expression thus largely await further investigation.

Regulation by ARF-BZR-PIF

The mechanisms controlling auxin, brassinosteroid, gibberellic acid (GA) and light-regulated *SAUR* expression have been largely elucidated in recent years. Oh et al. (2014) showed that ARF6, BZR1 and PHYTOCHROME INTERACTING FACTOR 4 (PIF4) can physically interact with each other in hypocotyls and have largely overlapping target gene sets, including a large number of *SAUR* genes. This points to a major role for an ARF-BZR-PIF complex in the regulation of *SAUR* gene expression. In line with this, *SAUR* genes can be synergistically upregulated by combined addition of auxin and brassinosteroids, (Bemer *et al.*, 2017b; van Mourik *et al.*, 2017; Walcher and Nemhauser, 2012), are abundantly present in target lists of ARF5, ARF7, ARF8 and ARF19 (Nagpal *et al.*, 2005; Okushima *et al.*, 2005; Schlereth *et al.*, 2010) and downstream of different PIFs (Sun *et al.*, 2016; van Mourik *et al.*, 2017). Several other studies have provided additional evidence for the role of a ARF-BZR-PIF complex in the SAUR-induced growth response. Sun et al. (2016) showed direct binding of PIFs to the *lirSAURs*, which induces their expression in dark-grown hypocotyls; Miyazaki et al. (2016) reported that the hypocotyl elongation phenotype of *LOV KELCH PROTEIN 2* (*LKP2*) overexpression is accompanied by *SAUR* gene upregulation and depends on both auxin and PIFs; and Favero et al. (2017) found that both brassinolide and auxin treatment enhanced transcript accumulation of *SAUR19* subfamily genes in hypocotyls and that blocking polar auxin transport could attenuate the growth responses of SOB3 mutants to exogenous brassinolide. Moreover, family-wide *in silico* analysis of the regulatory regions of the Arabidopsis *SAUR* genes revealed that inverted repeats of two AuxRE elements, bound by ARFs (Boer *et al.*, 2014), are enriched in auxin-induced Class I SAURs, in combination with BZR and PIF5 binding motifs (van Mourik *et al.*, 2017).

GA also plays a role in the ARF-BZR-PIF signalling module, as the growth-inhibiting DELLA proteins interact with BZR1 and with ARF6 (Bai *et al.*, 2012; Bemer *et al.*, 2017a; Oh *et al.*, 2014), thereby preventing their binding to the DNA. In the presence of GA, DELLAs are degraded and the ARF-BZR-PIF complex can induce *SAUR* expression. DELLAs can also interact with PIFs, thus controlling the activity of the inducing complex even more (De Bruyne *et al.*, 2014). In line with this, GA induced hypocotyl elongation requires both BZR1 and PIFs (Bai *et al.*, 2012). These data indicate that at least in Arabidopsis, there is a distinct group of *SAURs* that can be induced by auxin, brassinosteroids, gibberellin and light through ARF-BZR-PIF complexes (van Mourik *et al.*, 2017). The light response of *SAURs* is regulated via the PIFs, which are degraded by the phytochromes in the active Pfr state when the ratio of red to far-red light is high (Castillon *et al.*, 2007). In low light conditions or at low red:far-red ratios (in the shade), PIFs are active and induce *SAUR* expression. *SAURs* thus both regulate growth downstream of photomorphogenesis and contribute to the shade avoidance response (Ren and Gray, 2015; Sun *et al.*, 2016; van Mourik *et al.*, 2017). Also warm-temperature-induced *SAUR* upregulation in Arabidopsis is mediated by the PIFs (Franklin *et al.*, 2011). Research in other species have linked brassinosteroid and light signalling to *SAUR* gene expression as well. Mutations in the *Medicago* brassinosteroid receptor MtBRI1 affected the expression of a set of *SAUR* genes (Cheng *et al.*, 2017), a potato *SAUR* gene was identified as a marker for induction of the brassinosteroid pathway (Wiesel *et al.*, 2015), *SAURs* were identified downstream of PIFs in rice (Kudo *et al.*, 2017) and light treatments in Norway spruce also induced *SAUR* gene expression (OuYang *et al.*, 2015). In conclusion, the cell elongation capacity and light response of seedlings appears to a large extent regulated by the ARF-BZR-PIF complex.

Other factors involved in SAUR regulation

The regulatory networks involved in the repression of *SAURs* upon stress conditions such as cold, drought and increased salinity have been less well characterized. However, Kodaira et al. (2011) showed that the cold- and high salt-inducible TFs ARABIDOPSIS ZINC-FINGER 1 (AZF1) and AZF2, which function in the ABA response pathway, can repress 15 *SAUR* genes. Electrophoretic Mobility Shift Assays (EMSAs) also showed that both TFs can bind to the upstream region of *SAUR20* and *SAUR63*, indicating that the regulation of the *SAURs* by AZF1/2 occurs via direct binding. The repressive effect of JA is probably transduced via the

ARF-BZR-PIF complex, because JAZ proteins can interact with the DELLA proteins, thereby inhibiting the interaction of the DELLA proteins with the PIFs. In the presence of JA, JAZ proteins are degraded, resulting in increased DELLA-mediated inhibition of ARF-BZR-PIF (Yang *et al.*, 2012). The fact that the *pifq* mutant is impaired in JA-induced growth inhibition (Yang *et al.*, 2012), confirms this dependency of JA signalling upon the ARF-BZR-PIF complex.

SAUR transcript levels are also regulated in a circadian manner. The sunflower *SAUR50*-like gene for example, is particularly highly expressed in the morning at the east-side of the stem (Atamian *et al.*, 2016), while the circadian movement of waterlily flowers is under control of auxin, associated with day-time dependent expression of 25 *SAUR* homologs in the petals (Ke *et al.*, 2018). In Arabidopsis hypocotyls, *SAURs* are induced by PIFs (Oh *et al.*, 2014; Sun *et al.*, 2016), which accumulate at dawn in short-day (SD) seedlings (Soy *et al.*, 2014). This suggests that *SAUR* transcripts may also be most abundant around dawn, at least in SD conditions, in agreement with the timing of maximum hypocotyl elongation (Soy *et al.*, 2014). Indeed, *SAUR63* subfamily genes revealed to be diurnally expressed, with highest expression in the early morning (Chae *et al.*, 2012). The clock genes PSEUDO-RESPONSE REGULATOR 5 (PRR5) and PRR7 are negative regulators of hypocotyl growth expressed in the course of the day, and act as transcriptional repressors (Nakamichi *et al.*, 2010). Both factors can directly bind to many Arabidopsis *SAUR* genes (van Mourik *et al.*, 2017), thereby probably repressing their expression in the afternoon. Thus, the majority of the *SAUR* genes may be higher expressed in the early morning and repressed in the afternoon through the upstream control of clock genes. Family-wide temporal expression analyses are required however, to validate this circadian expression pattern.

In addition to upstream regulation of *SAUR* gene transcription, post-transcriptional and post-translational regulation of SAUR activity also contributes considerably to the SAUR-mediated dynamic growth control. *SAUR* overexpression gives a much more severe phenotype when fused to a tag such as GFP, which probably stabilizes the protein that has a very short half-life (Chae *et al.*, 2012; Knauss *et al.*, 2003; Ren and Gray, 2015). Besides the rapid protein decay, which has not been further investigated so far, several studies have shown that *SAUR* transcript levels quickly drop after removal of the inducer (e.g. auxin) (Markakis *et al.*, 2013; van Mourik *et al.*, 2017). This post-transcriptional regulation is at least in part regulated by a ~40-nucleotide downstream (DST) element in the 3' untranslated region (UTR) of a number of *SAUR* genes. This region was initially characterized in a few *SAURs* from soybean, mung bean

and Arabidopsis (McClure and Guilfoyle, 1989; Newman *et al.*, 1993), and confers mRNA instability (Newman *et al.*, 1993). Sullivan and Green (1996) identified two functionally important conserved regions within the DST element (ATAGAT and GTA) by mutational analysis in tobacco. The DST element, more precisely defined as GGA(N)xATAGAT(N)xGTA, is present in 30 of the 79 Arabidopsis *SAURs* (Ren and Gray, 2015). Overexpression of Arabidopsis *SAURs* including the DST element resulted in much less severe phenotypes than when the element was excluded (Hou *et al.*, 2013; van Mourik *et al.*, 2017). Putative DST elements were also identified in *SAURs* from rice (Jain *et al.*, 2006) and tomato (Wu *et al.*, 2012). The DST element has been associated with circadian control of mRNA, because several other transcripts with a DST sequence, which are upregulated in the *dst1* and *dst2* EMS mutants, are regulated in a circadian manner (Pérez-Amador *et al.*, 2001). However, more recently, also oxidative stress was found to induce transcript degradation via 3'UTR DST sequences (Ravet *et al.*, 2012), suggesting that several upstream cues can induce DST-mediated transcript degradation. Which upstream factors regulate *SAUR* mRNA decay remains to be investigated. Identification of the loci causal for the *dst1* and *dst2* molecular phenotypes would certainly contribute to the elucidation of DST-controlled *SAUR* mRNA decay.

The data summarized in this section illustrate the complex regulation of the *SAUR* genes via both developmental, environmental and clock-controlled pathways at the transcriptional and post-transcriptional levels (see Figure 3). Despite the high level of complexity, many regulatory modules appear to converge at the ARF-BZR-PIF complex, which integrates various upstream cues. In addition, tissue-specific TFs and other upstream regulators also contribute significantly to the dynamics of *SAUR* activity, and also determine the expression of the *SAURs* that are not regulated via ARF-BZR-PIF.

Concluding remarks

Land plants need to constantly adapt their growth to the environmental circumstances in accordance with their developmental stage. To achieve this, they evolved dynamic growth factors that can rapidly induce growth in response to a wide range of internal and environmental stimuli. These growth factors, the SAUR proteins, generally share a common function in repression of PP2C.D phosphatases, but their genes exhibit a great regulatory

region diversity, allowing tissue-specific and stimuli-specific expression patterns. This provides the plant with a great toolbox for growth adaptation. The high retention of *SAUR* genes after duplication indicates that expansion of this toolbox delivers an evolutionary advantage. In Arabidopsis, about ~70% of the *SAUR* genes is responsive to auxin and probably regulated by the ARF-BZR-PIF complex. The majority of these *SAURs* regulate cell elongation, at least in the seedling (Sun *et al.*, 2016), which is linked to interaction of their proteins with the plasma-membrane localized PP2C.Ds (D2, D5 and D6) (Ren *et al.*, 2018). Plasma-membrane localized SAURs are presumably the main determinants of cell elongation, at least in part regulated via the ARF-BZR-PIF module. SAURs that are localized to the cytosol at the other hand, could repress the cytosolic PP2C.D PPSL, thereby inducing senescence. Several SAURs, such as SAUR10 and SAUR36 (Bemer *et al.*, 2017b; Hou *et al.*, 2013), can both induce cell elongation and senescence, and are thus expected to localize both to the plasma membrane and the cytosol. Interestingly, several SAURs exhibit nuclear localization (Narsai *et al.*, 2011; Park *et al.*, 2007), and can possibly target the nuclear-localized PP2C.Ds: D1, D3 and D4 (Ren *et al.*, 2018). The nuclear-localized SAUR32, which has been characterized in detail (Park *et al.*, 2007), inhibits cell elongation and is not responsive to auxin, suggesting that nuclear-localized SAURs may fulfill a function different from promoting cell elongation, possibly linked to induction by other factors, such as cytokinin. *SAUR51,* expressed in meristematic cells, is also non-responsive to auxin, but upregulated by cytokinin (van Mourik *et al.*, 2017). Future experiments will have to elucidate whether the localization of SAUR proteins is indeed predictive for their function, and whether this also correlates with their response to particular stimuli. Other outstanding questions regarding SAUR regulation and molecular function (see Table 1) will hopefully be solved in future studies as well.

Supplementary data

Figure S1. Full version of the phylogenetic tree displayed in Figure 1. The colours of the clade correspond with the colours in Figure 1. The tree was generated in MEGA based on a hand-adjusted Bio-Edit alignment (Supplementary data file 1)

Data File S1. Alignment of all SAUR proteins used to generate the phylogenetic tree.

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Tables

Table 1. Outstanding questions

• Which protein motifs determine the intracellular localization of the SAUR proteins?
• Can SAURs also interact with other PP2C clades?
• Which protein motifs are required for the interaction with PP2C.Ds?
• Is the effect on senescence regulated via the interaction with PPSL?
• Are only plasma membrane localized SAURs involved in cell elongation?
• What is the biological function of the calmodulin binding SAURs?
• Does the predicted metal-binding capacity of some SAURs have a biological function?
• Which TFs are involved in tissue-specific *SAUR* expression?
• Which SAURs act redundantly in the different tissues?
• Which pathways are involved in the response of *SAURs* to abiotic stresses?
• Is the response to ARF-BZR-PIF linked to plasma membrane localization?
• How is the DST-mediated *SAUR* mRNA decay regulated?
• What is the reason for the short half-life of SAUR proteins?

Figures

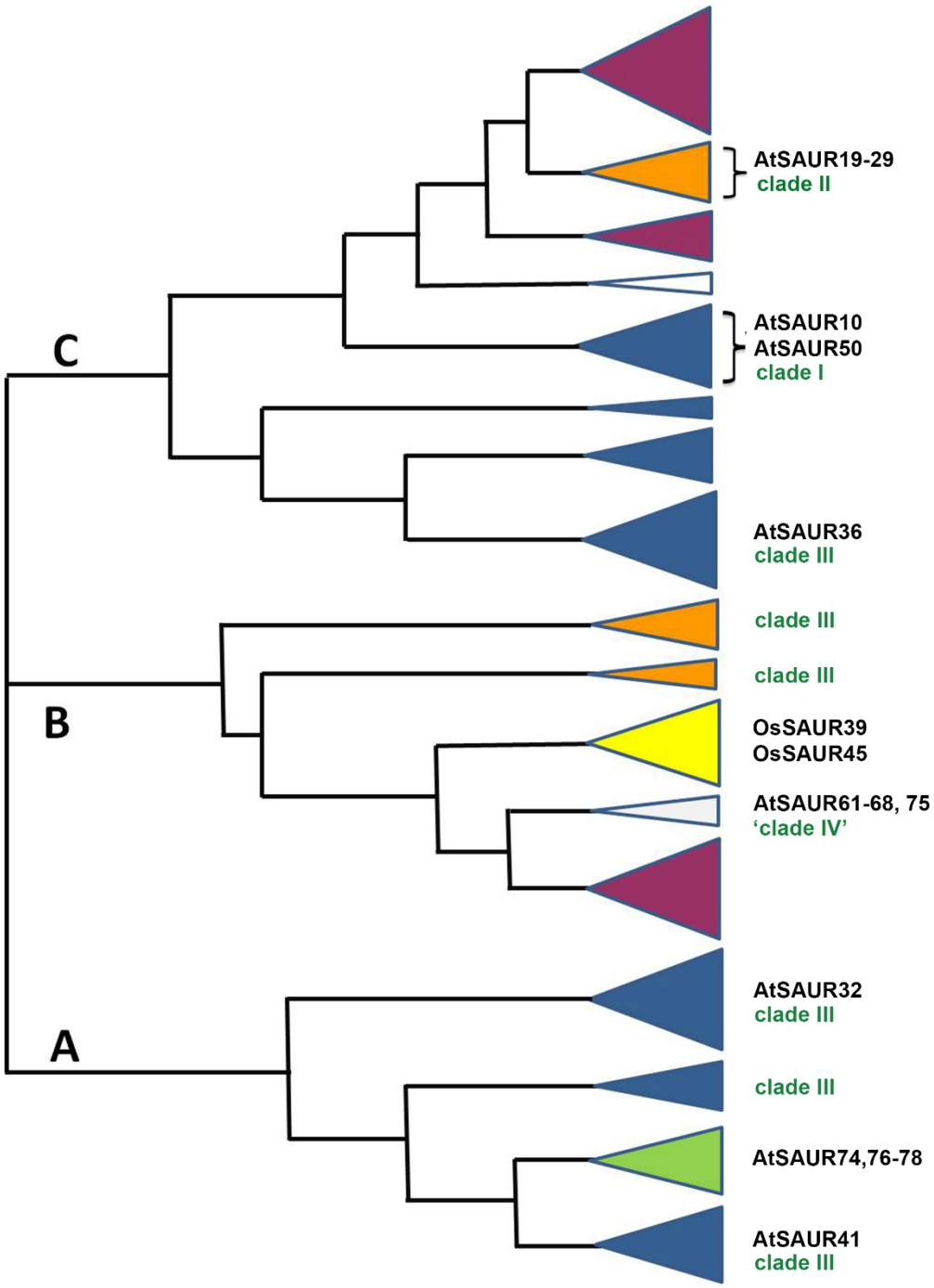

Figure 1. phylogenetic tree of the SAUR family. The unrooted tree was constructed from a hand-adjusted BioEdit alignment of all SAURs from Arabidopsis (www.Arabidopsis.org), Physcomitrella (Rensing *et al.*, 2008), potato (Wu *et al.*, 2012), tomato (Wu *et al.*, 2012), rice (Jain *et al.*, 2006) and sorghum (Wang *et al.*, 2010) (Supplemental data) using the maximum

likelihood method in the MEGA software (Hall, 2013). The colours of the triangles indicate the species represented in that clade. Green: all species (including Physcomitrella); Blue: eudicot and monocot; Yellow: monocot; Orange: eudicot; White: Arabidopsis; Purple: Solanum. In some cases, the separation of the clades is uncertain and supported by low bootstrap values (Supplemental Figure S1). Some characterized SAURs have been listed alongside the clades. The clade division from Kodaira et al. (2011) is indicated in dark green.

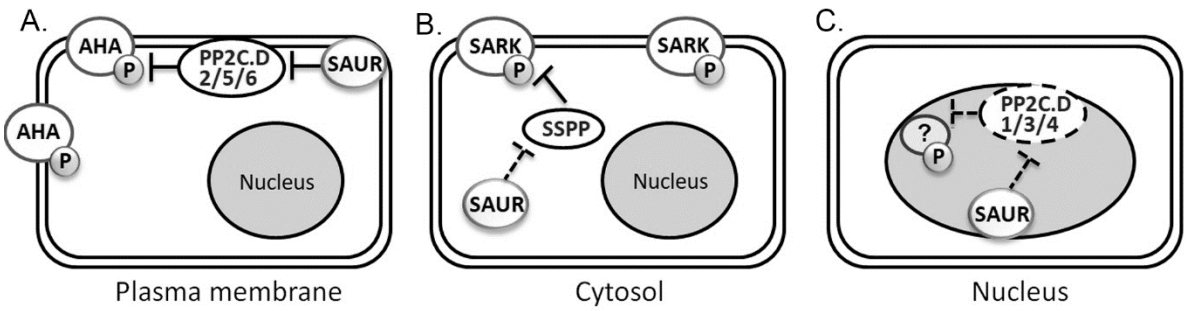

Figure 2. Schematic model of the putative molecular functions of SAURs in different cell compartments. A) In the plasma membrane, SAURs interact with PP2C.D2/5/6, thereby repressing dephosphorylation of the H+ATPases AHA1/2 and inducing cell elongation. **B)** In the cytosol, SAURs can probably interact with SSPP (PP2C.D1), thereby repressing dephosphorylation of AtSARK and inducing senescence. **C)** In the nucleus, the function of SAURs is still unclear, but they may interact with the nuclear localized PP2C.D1/3/4.

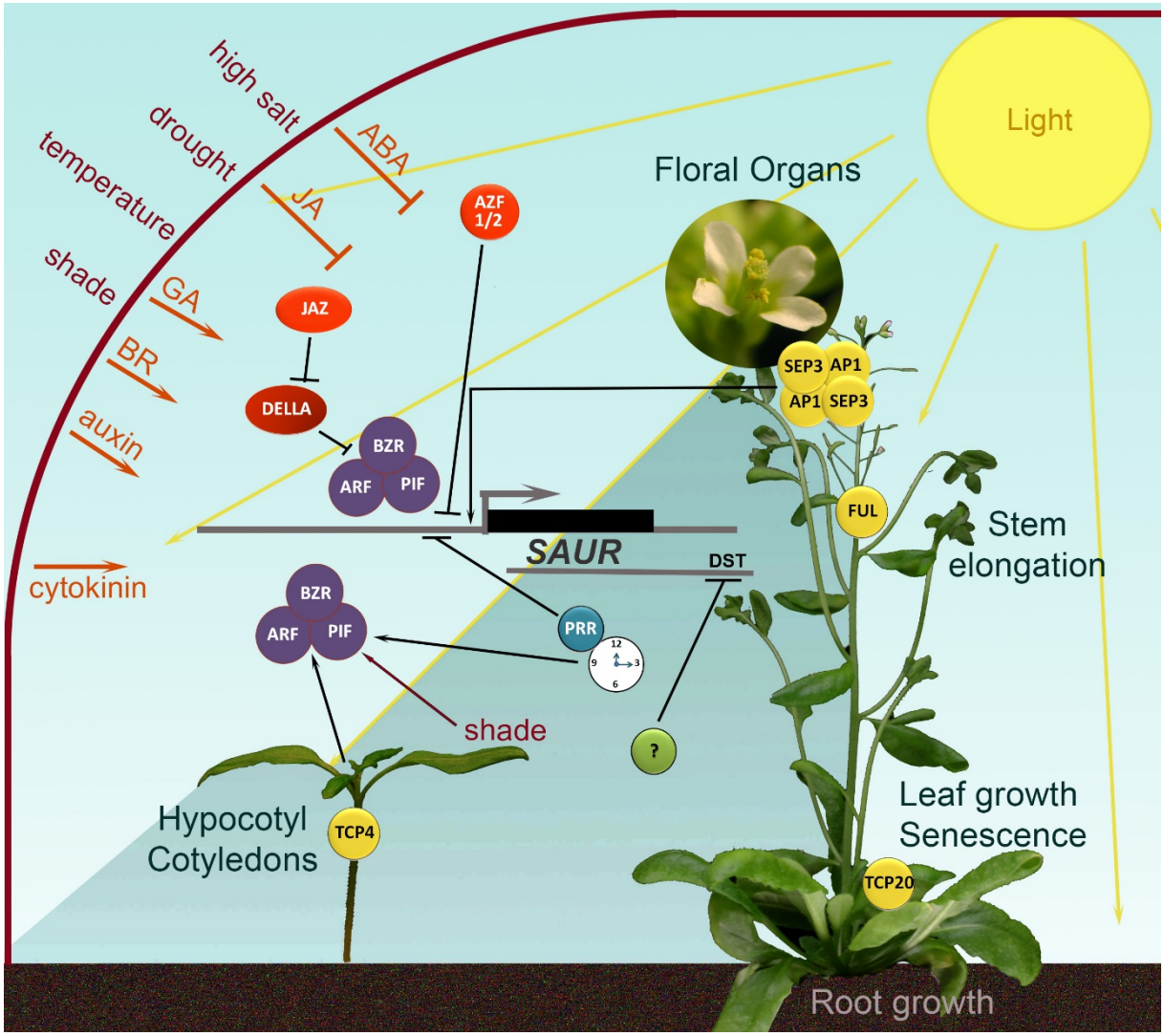

Figure 3. Regulation of SAUR genes by developmental, environmental and clock-controlled factors. The different tissues where *SAURs* play a role are indicated, as well as some upstream tissue-specific regulators (in yellow). Environmental signals (dark red) are transduced via hormones (orange). Most pathways converge at the level of the ARF-BZR-PIF complex (purple), while others directly act on the upstream region of *SAUR* genes or affect transcript stability. The black lines indicate direct or indirect activation or repression. The circadian regulation is indicated with a clock symbol.